08/486,313. These related applications were filed on June 7, 1995. Each application is the same and includes the specification of the present application.

Rejections under 35 U.S.C. § 112, 1st ¶

The Examiner objects to the specification under § 112, 1st ¶, because the specification does not describe the administration of the Bcl-2 protein. Claim 7 has been amended to clarify that the Bcl-2 gene is administered.

The Examiner also objects to the specification stating that "the specification is not enabling for the practice of the invention in humans and the claims must be limited to non-human mammals." The Examiner finds the specification lacking with respect to 1) sites of implantation of the mini-pumps, 2) the amount to administer, 3) the time course of administration, and 4) evidence of similarity between human and mouse cell types in the subependymal cells.

In related application U.S. Ser. No. 08/270,412 (which is a continuation of U.S. Ser. No. 07/726,812, referenced on p. 1, line 4 of the present application), applicants provided declaratory evidence that neural stem cells were obtained from adult human tissue and cultured *in vitro*. Attached hereto as Exhibit A is a copy of that declaration. Paragraphs 7-9 of the declaration discuss the culture of human neural stem cells obtained from tissue surrounding the lateral ventricle and *in vitro* proliferation of those in the presence of growth factors. The present specification teaches that the techniques that are used to proliferate and differentiate CNS stem cells *in vitro* can be adapted to achieve similar results *in vivo* techniques (see p. 13, lines 20 et seq.).

Regarding the sites of implantation of mini-pumps, the specification provides extensive disclosure concerning the location of stem cells in mammalian neural tissue (e.g. p. 13, line 27 to p. 18 line 7). As stated in the specification:

> "the ventricular system is found in nearly all brain regions and thus allows easier access to the affected areas. If one wants to modify the stem cells by exposing them to a growth factor or a viral vector it is relatively easy to implant an osmotic pump or make injections into the ventricles."

Thus, the specification is clear that a CNS ventricle is a suitable site for the
implantation of a mini-pump. The ventricular system described on page 17, lines 12-16 pertains to human CNS anatomy as well as mice.

Regarding the amount of growth factor to administer and the duration/length of treatment, as noted above, the specification teaches that the techniques used to proliferate CNS stem cells *in vitro* are adaptable to *in vivo* techniques. Example 2 discloses the *in vitro* proliferation of mouse neural stem cells in the presence of 20 ng/ml EGF. Example 1 describes the extent of *in vivo* proliferation of mouse neural stem cells induced by the infusion of 3.3 - 330 μg/ml EGF from a 0.5 μl/hour osmotic mini-pump into the lateral ventricles for 6 days. Thus, from the information provided in the specification, one of ordinary skill in the art would know the approximate amount of growth factor to administer to achieve stem cell proliferation *in vitro* and *in vivo*. Suitable ranges of growth factor concentration and optimal levels for a particular ventricular

region of a particular mammal could be readily ascertained by routine experimentation.

The Reynolds Declaration (Exhibit A) demonstrates that human neural stem cells proliferated *in vitro* in the presence of 20 ng/ml EGF and bFGF. The same concentration of growth factor results in the *in vitro* proliferation of mouse neural stem cells (see p. 7, lines 2-7). Therefore, it is expected that since the same procedures result in the proliferation of mouse and human neural stem cells *in vitro*, a procedure that induces the *in vivo* proliferation mouse neural stem cells *in vivo* could be used to induce *in vivo* proliferation of human neural stem cells. As alluded to by the Examiner at the bottom of page 3 of the office action, standard dosage considerations, including the weight of the recipient would of course be factors to consider in adapting a protocol for a human recipient. However, these are parameters that would be fine-tuned during a clinical evaluation of the treatment. Applicants are not required to submit such data to establish patentability (see MPEP § 2107.02 and cases cited therein).

Regarding the Examiner's concern as of lack of evidence of similarity between human and mouse cell types in the subependymal cells, it is believed that the Reynolds Declaration attached as Exhibit A adequately addresses this concern by showing that precursor cells can be obtained from human neural tissue and cultured and proliferated *in vitro* using the same techniques used for mice. Further support for this assertion is provided in another Reynolds Declaration attached hereto as Exhibit B which was originally provided in related application U.S. Ser. No. 07/726,812. Paragraph 4 of that declaration discusses the *in vitro*

proliferation and differentiation of neural stem cells (referred to in the specification as progenitor cells) from species other than mice. The same techniques that are used to induce proliferation of mouse neural stem cells induces the proliferation of neural stem cells from other mammalian species. The techniques described in the declaration are the same as those described in the present specification as well as the related applications (which have been incorporated by reference into the present application).

The data discussed in the declarations concerns *in vitro* proliferation of human neural stem cells. Attached hereto is a facsimile copy of a new declaration dated 6 May 1996 from Dr. Reynolds concerning the *in vivo* proliferation of neural stem cells in primates. As stated in the declaration, "the precursor cell distribution in adult primate CNS appears to closely mimic that found in mice." This was demonstrated by BrdU labeling studies in adult rhesus monkeys (discussed in ¶s 4A to 4J of the declaration) which were similar to the experiments described in the specification using mice (see p. 14, lines 12 et seq.). The new Reynolds Declaration also demonstrates that the precursor cells of the subventricular zone of primate lateral ventricle are responsive to growth factors. Similar to the mouse studies, "growth factor infusion into the adult primate brain results in a dramatic increase in the endogenous subependymal neural precursor cell populations and stimulates their migration away from the lateral ventricle walls into adjacent normal brain parenchyma (¶ 5H of declaration).

Thus, applicants have provided overwhelming evidence of the similarity between primates (including human) and mouse cell types in the subependymal cells. Accordingly, it is believed that the

Examiner's rejection under § 112, 1st paragraph should be withdrawn.

The claims that use the terminology "neurological disorder" have been canceled. Accordingly, the Examiner's arguments raised on pages 4 and 5 are no longer applicable to the claims presently under examination. However, Applicants would like to address the Examiner's discussion of the Emerich paper as it illustrates some important distinctions between Applicants' invention and the prior art. The Examiner quoted the Emerich *et al.* paper to support the argument that the specification is not enabling for the practice of the invention in humans. While Emerich *et al.* point out cases where results of transplantation of tissue caused problems, applicants' invention is designed to overcome some of these problems by inducing the patient's own cells to promote recovery without the need for transplantation. The problems associated with the administration of drugs such as L-dopa, outlined by the Examiner on page 5 of the office action, are the reasons why applicants' technology is a significant advancement over the prior art. The patient's damaged or lost dopaminergic cells are replaced by new dopaminergic cells and would be under the control of the patient's own body, thus reducing the side-effects seen with the administration of L-dopa. This has been discussed in the co-pending applications referenced above (at p. 44 line 13 et seq.) and is reproduced below:

> Normally the cell bodies of dopaminergic neurons are located in the substantia nigra and adjacent regions of the mesencephalon, with the axons projecting to the striatum. Prior art methods for treating Parkinson's disease usually involves the use of the drug L-Dopa, to raise dopamine levels in the striatum. However, there

are disadvantages with this treatment including drug tolerance and side effects. Also, embryonic tissues that produce dopamine have been transplanted into the striatum of human Parkinsonian patients with reasonable success. However, the use of large quantities of fetal human tissue required for this procedure raises serious ethical concerns and practical issues.

The methods and compositions of the present invention provide an alternative to the use of drugs and the controversial use of large quantities of embryonic tissue for treatment of Parkinson's disease. Dopamine cells can be generated in the striatum by the administration of a composition comprising growth factors to the lateral ventricle. A particularly preferred composition comprises a combination of EGF, FGF-2, and heparan sulphate. The composition preferably also comprises serum. After administration of this composition, there is a significant increase in the transcription of messenger RNA (mRNA) for TH in the subventricular region of the striatum, an area which normally does not contain dopaminergic cell bodies. These methods and results are described in detail in Example 34. As detailed in Example 35, the use of dual labeling tissue to show the distribution of BrdU+ and TH+ cells indicates that, in response to the *in vivo* administration of growth factors, TH+ cell bodies occur in striatal tissue. Many of these newly generated TH+ cells are also BrdU+

Rejections under 35 U.S.C. § 103

Applicants acknowledge the duty to disclose possible 102(f) or (g) art under 37 CFR § 1.56. The invention(s) defined by the claims of the present application were commonly owned when made.

The Examiner has objected to claims 1, 2 and 4-6 under § 103 as being unpatentable over Morshead *et al.* and Smart taken

with Williams *et al.* and Morrison *et al.* This application is a continuation-in-part of parent application U.S.S.N. 07/726,812 (filed July 8, 1991; the contents of which have been incorporated by reference). The priority application discloses inducing *in situ* proliferation of precursor cells using exogenous growth factors such as epidermal growth factor (see p. 14, lines 2-4; p. 18, lines 21-24; p. 32, lines 9-24; claims 22 and 23), and that growth factors can be administered *in situ* using timed-release apparati (e.g. osmotic pumps; see p. 32, lines 19-24). The priority application also discloses that precursor cells are located in ventricular tissue (see p. 20, line 13 and Example 13).

Morshead *et al.*, the primary reference cited by the Examiner, is used to show that it was known in the prior art that stem cells are present in the subependymal layer. Without agreeing to the Examiner's characterization of this reference, Applicants point out that the reference was not published until after the July 8, 1991 priority date of the present application which discloses the presence of EGF-responsive stem cells in ventricular tissue (e.g. the subependymal layer). Accordingly, Morshead *et al.* is not a proper reference for the purposes used by the Examiner and the rejection of Claims 1, 2 and 4-6 under § 103 over this reference should be withdrawn.

As early as 1961 (Smart), the presence of mitotically active cells in the subependymal layer of the adult brain was known. However, there is no evidence that the cells described by Smart are **stem cells**. In fact, prior to applicants' discoveries, it was not known that stem cells exist in the adult brain. Highly skilled neurobiologists have expressed surprise over applicants'

discoveries. Attached as Exhibit C is an article from The New York Times, (March 27, 1992), which demonstrates that researchers in the field of neurobiology were of the belief, at the time of the invention, that neural stem cells were not found in the adult mammalian CNS. Note the comment made by Dr. Emmanuel DiCicco-Bloom: "It wasn't thought possible that you would find this (stem cells that can proliferate and produce neurons) in the mature mammalian brain". The comments made by various researchers in the field of neuroscience which are quoted in this article provide compelling secondary evidence of the unobvious nature of the claimed invention.

Returning to the Smart reference, the Examiner notes that the cells labeled by Smart had a high rate of mitotic activity, with a turnover time of 4-15 hours (page 336 of Smart). This description does not fit that of neural stem cells, which are normally quiescent and have a turn over time that is measured in days, not hours. It is likely that Smart labeled the "constitutively proliferating cells" described in Morshead *et al.* and in the specification (p. 13-14), which have a similar turnover time to that described by Smart. As indicated in the specification (Example 6 and p. 15, line 24 to p. 16, line 30), the constitutively proliferating cells are a population of cells which, while derived from stem cells, are clearly distinct from the neural stem cell population. Thus, the Smart paper does not disclose the presence of growth factor-responsive neural stem cells in adult tissue.

As the Smart paper is not relevant to applicants' invention and as the Morshead paper is cited for disclosing what was already disclosed in the priority application, it is believed that the rejection

of claims 1, 2 and 4-6 under § 103 are overcome. Also, the remaining § 103 rejections are also overcome as they primarily rely on these two references. However, for the sake of completeness, applicants briefly discuss below why the other papers cited by the Examiner are not relevant to the present invention.

The Examiner cited Morrison *et al.* for the teaching that EGF "enhances ***survival*** of neonatal rat brain cells in a dose dependent manner" (p. 7 of office action, 6 lines from bottom; emphasis added). However, merely promoting cell survival is not the subject of applicants' claimed invention. Applicants' invention entails the use of growth factors to ***induce proliferation*** of precursor cells. The mere fact that a cell has receptors for EGF does not mean that the cell will be induced to proliferate in the presence of EGF. On page 8, line 7, the Examiner states that Morrison teaches that "EGF stimulates the proliferation and differentiation of CNS cells." However, at no point in this reference does Morrison imply that this is the case with respect to neural stem cells. There is one statement in the paper that "EGF stimulates the proliferation and differentiation of glial cells" (page 72, second column). However, as glial cells are terminally differentiated cells which normally undergo mitotic activity, they are distinct from normally quiescent neural stem cells.

The Examiner relied on Cattaneo to teach that bFGF induces proliferation and/or survival of nestin positive cells and that nestin positive cells are stem cells. However, the Cattaneo reference does not describe the proliferation of neural stem cells, which, as described in applicants' specification, are capable of producing progeny that differentiate into neurons and glia. While nestin is a

marker for undifferentiated neural cells, ***nestin cannot distinguish a neural stem cell from a committed progenitor cell.*** Applicants studies have found that only about 1% of cells from a primary striatal culture are neural stem cells.

Thus, while the majority (95%) of the cells in Cattaneo's primary culture were undifferentiated, only a small percentage (approx. 1%) of those would be expected to be neural stem cells. The remainder of the nestin positive cells are most likely to be committed progenitor cells (i.e. undifferentiated cells capable of a limited amount of proliferation prior to differentiation). There is no evidence that neural stem cells, if present in Cattaneo's primary cultures, proliferated. It is possible that multipotent stem cells, if present in Cattaneo's cultures, died or survived the culture conditions but remained quiescent (i.e did not proliferate). The fact that Cattaneo's proliferated cells differentiated into **only** neurons, indicates that Cattaneo's culture method, which differed from Applicants' method in several aspects (including exposure of the cells to serum), did not induce proliferation of multipotent neural stem cells. Instead, the results indicate that Cattaneo's culture methods induced the proliferation of a unipotent neuronal progenitor cell.

In summary, the fact that neuronal progenitor cells of Cattaneo were nestin+ does not indicate that they were neural stem cells, it merely means that the cells identified in the Cattaneo culture were undifferentiated.

With regards to claims 29-31, none of the references cited by the Examiner, nor a combination of the references suggests that prior to *in vitro* proliferation, stem cells can be "primed" *in vivo* by

the administration of growth factors to the ventricles. Applicants have found that this substantially increases the numbers of cells that can be proliferated *in vitro* - presumably because the stem cells present *in vivo* are induced to proliferate, producing a larger number of stem cells per volume of tissue removed. The tissue is then harvested and the larger number of stem cells produces more progeny.

CONCLUSIONS

For the foregoing reasons, it is believed that the specification adequately describes and enables the claimed invention and that, at the time the invention was made, it was unobvious in view of the prior art.

Respectfully submitted,

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